The Influence of Four Unnecessary Genes for Virulence on the Fitness of *Erysiphe graminis* f. sp. *tritici*

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ABSTRACT

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Two isolates of *Erysiphe graminis* f. sp. *tritici* that differed in their host range were examined for possible evidence in support of Vanderplank's stabilizing selection hypothesis. Isolate MO-10 was virulent and isolate MS-1 avirulent on wheat line CI 15889 and lines with the *Pm*2, *Pm*3a, and *Pm*4 resistance genes. Genetic analysis of the isolates showed one locus controlling each difference in host range. A mix of the two isolates was grown on cultivar Chancellor (on which both isolates were virulent) in a controlled environment and the relative proportions of each isolate were monitored over successive conidial generations. The frequency of MO-10 decreased and the frequency of MS-1 increased, indicating that MO-10 was less fit than MS-1 under these conditions ($s_x = 0.24$). However, when F_1 progeny of the isolates were compared in 8-way mixtures, reduced fitness was found to have segregated independently of the identified virulence loci. The results indicated that the fitness of the progeny was not significantly affected by the unnecessary virulence alleles. No evidence was found to support the hypothesis of stabilizing selection.

Additional key words: powdery mildew, selection coefficient.

Vanderplank (20,21) postulated that natural selection tends to eliminate pathogen races with unnecessary genes for virulence. This directional selection presumably stabilizes the population and prevents the accumulation of virulence genes. If this phenomenon is common, our ability to control plant disease through the use of various gene management strategies (such as gene recycling, multilines, cultivar mixtures, and multigene cultivars) may be enhanced (6). However, the validity and general applicability of Vanderplank's hypothesis has not been established (3,4,9, 10,11,14,19,22,23).

Our objective was to examine two isolates of *Erysiphe graminis* D.C. f.sp. *tritici* E. Marchal, causal agent of powdery mildew of wheat, that were known to differ in host range, and to determine if any differences in their fitness were due to unnecessary virulence genes. The approach was to compare the ability of the two isolates to compete in a mixture, to determine the genetic control of the differences in host range, and to determine if reduced fitness in the F_1 progeny of the isolates segregated with unnecessary virulence alleles.

MATERIALS AND METHODS

Plant material. Seeds of the differential wheat lines used in this study (Table 1) were provided by R. A. Kilpatrick, Beltsville Agricultural Research Center, Beltsville, MD. With the exception of PI 367698, all lines were developed by backcrossing powdery mildew resistance genes from various wheat cultivars into the susceptible cultivar Chancellor (1,16). Except for plants used to cross the fungal isolates (see below), seedlings were grown in soil in small containers (237-ml paper cups) and inoculated 7 days after planting. Seed lots of cultivar Chancellor (CI 12333) were tested for off-type (resistant) seed by inoculating seedlings (growing in a controlled-environment chamber) with conidia and observing infection type after 7 days. Among 976 plants inoculated with isolate MS-1 and 245 inoculated with isolate MO-10, no resistant plants were detected.

Fungal cultures. Two isolates and their F_1 progeny were used in this study. MS-1 was isolated in 1961 as a greenhouse contaminant. MO-10 was isolated in 1976 from a Michigan powdery mildew nursery on a wheat line with resistance gene *Pm*4. MO-10 was selected for use because it had a wider virulence spectrum than MS-1 and crossed readily with it. The isolates were maintained in separate controlled-environment chambers as described elsewhere (13).

The isolates were crossed by inoculating a mixture of MS-1 and MO-10 on 6-wk-old plants (cultivar Chancellor) growing in 25-cm-diameter pots in a controlled-environment chamber with fluorescent (2.5×10^4 ergs·sec^{-1}·cm^{-2}) and incandescent (1.5×10^4 ergs·sec^{-1}·cm^{-2}) lighting. Temperature in the chamber was 20 ± 1 C during the day (14 hr) and night (10 hr) cycles. After the first appearance of cleistothecia at 2 wk, the day temperature was raised by 0.5 C per day to a final temperature of 26 C. Growth of the fungus was inhibited at this temperature, thus preventing premature death of the plants. This step was not necessary for production of cleistothecia capable of releasing viable ascospores (15; and C. R. Bronson, *unpublished*). *E. graminis* is obligately heterothallic (5); thus, cleistothecia formed were assumed to be the result of hybridization. Ascospore formation and release were induced by incubating the cleistothecia at room temperature on damp filter paper suspended above excised wheat leaves floating on a benzimidazole solution (20 ppm) (17). Colonies that developed were assumed to be mixtures and were purified to a single genotype by at least two cycles of single-colony isolation (see below). A total of 162 progeny were obtained from this cross.

Progeny were stored at 6 ± 2 C on Chancellor seedlings growing in vermiculite in 2.5×30.5-cm glass test tubes with foam rubber plugs. Continuous light was provided by a 15-W white fluorescent bulb. Cultures were transferred at 4- to 6-wk intervals.

Cultures were purified immediately prior to use by the isolation of single colonies. Seedlings were lightly dusted with conidia. Leaf pieces bearing isolated colonies were excised 3 days after inoculation and floated on water for 3 days. This permitted further growth and sporulation of the colony and the die-off of conidia dislodged during excision (2). Conidia from these colonies were

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then either used immediately or dusted onto another seedling for a second cycle of purification.

Determination of virulence spectra and mating types. The virulence spectra of the isolates were determined by inoculating wheat seedlings (10–15 of each line) with conidia and observing the infection type after 7 days. Infection was rated on a progressive scale from 0 to 4, with 0 indicating no macroscopically observable fungal growth or host response and 4 indicating heavy sporuation (Table 1). Tests were performed under both greenhouse and laboratory conditions. In the laboratory, the seedlings were covered with glass lamp chimneys before and after inoculation and placed under Gro-Lux lights (10^4 ergs·sec^{-1}·cm^{-2}). The tops of the lamp chimneys were covered with a double layer of tissue paper to prevent cross-contamination. Temperature within the chimneys was 24 ±1 C during the light cycle (14 hr) and 21 ±1 C during the dark cycle (10 hr). In the greenhouse, tests were performed three separate times using coded treatments to permit blind rating. The seedlings were not covered except during the inoculation procedure and for 12 hr thereafter. Uninoculated controls were included to monitor contamination, which was rare. Progeny mating types were assigned to strains based on their ability to form cleistothecia with the parental isolates when paired on wheat seedlings. Cleistothecia formed only when paired with a parent strain of the opposite mating type.

Determination of relative fitness and estimation of selection coefficients. Relative fitness of each isolate was determined by monitoring its change in frequency in a mixed population over successive conidial generations in growth chambers. Parental isolates were compared in 2-way mixtures (12, 8, and 6 generations for the first, second, and third replications, respectively). Progeny were compared in three sets of 8-way mixtures (16 generations). Each progeny set included eight isolates, one representative of each of the eight possible virulence patterns on wheat lines with resistance genes *Pm*2, *Pm*3a, and *Pm*4. Discrete (nonoverlapping) generations of mildew were obtained by a sequence of addition and removal of pots of seedlings (cultivar Chancellor) from the chambers. The first set of seedlings was inoculated when 6 days old with approximately equal frequencies of conidia of the isolates. Starting on the third day after inoculation and every 7 days thereafter seedlings (25–30 per pot, 10 pots per chamber) were placed in the growth chambers (2 days after planting and prior to emergence). Starting on the 10th day and every 7 days thereafter (16 days after planting and prior to the appearance of secondary infection), 10 pots were removed. Conidia were dispersed by the air currents in the chambers resulting in one fungal generation per week. The population size (number of colonies in each generation) was generally over 2,500 in the initial generation and over 10,000 in succeeding generations. Each mixture was compared in three controlled-environment chambers (Sherer-Gillett CEL 37-14 and CEL 25-7HL) with fluorescent (2.5×10^4 ergs·sec^{-1}·cm^{-2}) and incandescent (1.5×10^4 ergs·sec^{-1}·cm^{-2}) lighting. Temperature within the chambers was 20 ±1 C during the light (14 hr) and dark (10 hr) cycles.

The frequencies of the isolates were estimated every other generation (except for the frequencies of isolates in progeny set 3, which were estimated every fourth generation) by determining the genotypes of a random sample of approximately 100 conidia per sampling time per chamber. Approximately 10 leaves with sporulating infections were removed from the chambers and used to inoculate seedlings (cultivar Chancellor) by shaking the leaves over the seedlings in a settling chamber. The seedlings were placed under lamp chimneys in the lab and, after 3 days, well-isolated colonies were excised and purified. Infection types were determined under laboratory conditions.

Selection coefficients were estimated by fitting data to a selection model for asexually reproducing organisms undergoing discrete (nonoverlapping) generations in a complex mixture (2). A selection coefficient (s) was defined as $1 - W$ in which W (relative fitness) was the number of successful conidia produced per parent colony per generation for an isolate divided by the number of successful conidia produced per parent colony per generation for a reference isolate (either the most fit isolate or one of the most fit isolates in the mixture). A successful conidium was defined as one that infects and produces a daughter colony. Data were plotted as $\ln(q/p)$ versus generation in which q was the frequency of the less fit strain and p was the frequency of the reference strain. If s is constant, data plotted in this manner yield a straight line with a slope of $\ln W$. This method is identical to the method of Leonard (8) except that more than two isolates could be present and therefore one could not assume that $p + q = 1$. For a mixure of n isolates, $n - 1$ selection coefficients can be estimated, the fitness of the reference isolates having been defined as 1.0.

RESULTS

Phenotypes of MS-1, MO-10, and their progeny. The parental cultures (MS-1 and MO-10) differed in infection type only on CI 15889 and wheat lines with resistance genes *Pm*2, *Pm*3a, and *Pm*4 (Table 1). MS-1 displayed reduced virulence on CI 15889 that was detectable under greenhouse conditions but not laboratory conditions. MO-10 was virulent on wheat lines with *Pm*4 but less virulent than on Chancellor. Incomplete virulence on wheat lines with *Pm*4 has been reported previously (12,18). For each differential line, the F_1 progeny gave infection types that were identical to those of one or the other of the parental lines. Each test was performed three times with identical results.

Genetic control of virulence and mating type. A virulence locus was defined for this work as any locus that conditions a detectable difference in infection type. The virulence allele conditioned the higher infection type and the avirulence allele conditioned the lower infection type. Thus, if infection type segregated in the progeny, isolates giving the higher infection type were considered virulent and isolates giving the lower infection type were considered avirulent. Note that by this criterion the absolute infection type may not necessarily indicate virulence or avirulence. For example,

TABLE 1. Infection types of isolates of *Erysiphe graminis* f. sp. *tritici* on 20 differential wheat lines 7 days after inoculation of seedlings in the greenhouse

Wheat line	Resistance gene	Infection types[a]		
		MS-1	MO-10	F1
CI 12333[b] (Chancellor)		4	4	4
CI 14114	*Pm*1	0,1	0,1	0,1
CI 14115	*Pm*1	0,1	0,1	0,1
CI 14116	*Pm*1	0,1	0,1	0,1
CI 14117	*Pm*1	0,1	0,1	0,1
CI 14118	*Pm*2	2	4	2 or 4
CI 14119	*Pm*2	2	4	2 or 4
CI 14120	*Pm*3a	0,1	4	0,1 or 4
CI 14121	*Pm*3b	0,1	0,1	0,1
CI 14123	*Pm*4	0,1	3$^-$	0,1 or 3$^-$
CI 14124	*Pm*4	0,1	3$^-$	0,1 or 3$^-$
CI 14189	-[c]	4	4	4
CI 15520	-	3chl	3chl	3chl
CI 15886	-	4	4	4
CI 15887	-	0,1	0,1	0,1
CI 15888	-	4	4	4
CI 15889	-	3	4	3 or 4
CI 17739	-	3$^-$,3	3$^-$,3	3$^-$,3
CI 17760	-	3$^-$,3	3$^-$,3	3$^-$,3
PI 367698[d]	-	0,1,3$^=$	0,1,3$^=$	0,1,3$^=$

[a] Symbolism used to describe infection types: 0 = no symptoms; 1 = small, light-green flecks with no sporulation; 2 = distinct necrotic and chlorotic spots with no or scant sporulation; 3$^=$ = few colonies with scant sporulation; 3$^-$ = scant sporulation; 3chl = reduced sporulation with a definite chlorosis; 3 = reduced sporulation; 4$^-$ = slightly reduced sporulation; and 4 = heavy sporulation, no chlorosis. Infection types were often higher in laboratory tests. MS-1 commonly gave infection types 3$^=$ on CI 14123 and CI 14124 and 4 on CI 15520, CI 15889, CI 17739, CI 17760, and PI 367698. MO-10 gave infection types 4$^-$ on CI 14123 and CI 14124 and 4 on CI 15520, CI 17739, CI 17760, and PI 367698.

[b] Cereal Investigations accession number.

[c] A dash indicates that the resistance gene has not been assigned a name.

[d] Plant Introduction number.

on host lines with the *Pm*4 gene, isolates giving a 3⁻ reaction under greenhouse conditions were considered virulent. However, on CI 15889, isolates giving a 3 reaction, which indicates more sporulation than a 3⁻, were classified as avirulent.

Genetic analysis of the inheritance of virulence (Table 2) gave segregation ratios that were not significantly different from 1:1, suggesting that a single locus was responsible for each difference in virulence between the two parental isolates. Mating-type ratios were also not significantly different from 1:1. Differences in virulence in the isolates assorted independently indicating that there were four distinct loci; no evidence was found for linkage among them. Locus *P*2, however, appeared to be linked to the locus controlling mating type at a distance of 16.7 map units (4.8 − 36.4, $P = 0.05$).

TABLE 2. Segregation of alleles for virulence in the F_1 progeny of isolate MS-1 and MO-10 of *Erysiphe graminis* f. sp. *tritici*[a]

Locus	Progeny (no.) Avirulent	Virulent	Ratio	χ^2	*P*
P2	70	92	1:1	2.72	0.09–0.10
P3a	71	91	1:1	2.23	0.10–0.25
P4	78	84	1:1	0.15	0.50–0.75
P15889	13	11	1:1	0.04	0.75–0.90

[a] Mating type segregated 10 mat+ : 14 mat− [χ^2 (1:1) = 0.38, P = 0.50–0.75].

TABLE 3. Selection coefficients for isolates MS-1 and MO-10 of *Erysiphe graminis* f. sp. *tritici* and their F_1 progeny

Isolate	Genotype	Selection coefficient[a]	S.D.[b]
Parents			
MS-1	*P2 P3a P4 P15889 mat+*	0.00[c]	···
MO-10	*p2 p3a p4 p15889 mat−*	0.24	0.07
Progeny set 1			
#52	*p2 p3a p4 p15889 mat−*	0.21	0.05
#59	*P2 p3a p4 p15889 mat+*	−0.01[d]	0.07
#70	*p2 P3a p4 p15889 mat−*	0.06	0.03
#65	*p2 p3a P4 p15889 mat−*	0.19	0.04
#92	*P2 P3a p4 P15889 mat−*	0.01	0.02
#51	*p2 P3a P4 p15889 mat−*	0.00	···
#78	*P2 p3a P4 P15889 mat+*	0.20	0.06
#89	*P2 P3a P4 p15889 mat+*	0.21	0.08
Progeny set 2			
#116	*p2 p3a p4 P15889 mat−*	0.17	0.08
#105	*P2 p3a p4 P15889 mat+*	0.18	0.04
#117	*p2 P3a p4 P15889 mat−*	0.30	0.07
#121	*p2 p3a P4 P15889 mat−*	0.10	0.08
#102	*P2 P3a p4 p15889 mat+*	0.09	0.08
#124	*p2 P3a P4 p15889 mat−*	0.00	···
#125	*P2 p3a P4 P14889 mat+*	0.20	0.01
#138	*P2 P3a P4 P15889 mat+*	0.16	0.03
Progeny set 3			
#146	*p2 p3a p4 p15889 mat−*	0.10	0.04
#150	*P2 p3a p4 P15889 mat−*	0.29	0.02
#106	*p2 P3a p4 P15889 mat+*	0.00	···
#141	*p2 p3a P4 P15889 mat−*	0.06	0.04
#143	*P2 P3a p4 P15889 mat+*	0.19	0.04
#127	*p2 P3a P4 p15889 mat−*	0.17	0.06
#140	*P2 p3a P4 p15889 mat−*	0.28	0.03
#149	*P2 P3a P4 P15889 mat+*	0.06	0.03

[a] Average of three replicates.
[b] Standard deviation.
[c] Isolates used as the standard for calculation of selection coefficients in each set were defined as having a selection coefficient of zero.
[d] Isolate #51, rather than #59, was chosen the standard for calculation of selection coefficients in this set because of its high fitness and relatively high frequency (and, thus, relatively small sampling error). Isolate #59 may have been slightly more fit than #51, as indicated by the negative selection coefficient, but the difference was not significant.

Based on these results, genotypes were assigned to the parent and progeny isolates (Table 3). Loci controlling virulence were given genetic symbols based on either the name of the corresponding resistance locus in wheat or the wheat line's accession number. Alleles for virulence were given the gene symbol *p*x in which x indicates the locus and alleles for avirulence were given the symbol *P*x. This symbolism is not intended to indicate dominance or recessiveness as dominance characteristics for virulence loci in *E. graminis* are not known. The mating type of MS-1 was arbitrarily designated "+." Thus, alleles at the mating-type locus were given the symbols *mat*− (indicating the ability to cross with MS-1) and *mat*+ (indicating the ability to cross with MO-10).

Relative fitness and its relationship to unnecessary virulence genes. A preliminary study revealed no difference in fitness between MS-1 and MO-10 (2). However, when the experiment was repeated using the environmental conditions described, a clear difference in fitness was detected. Isolate frequency changes for a representative mixture study are shown in Fig. 1. As MS-1 was the more fit isolate, the fitness of MO-10 was calculated in reference to it and this reduced fitness was expressed as a selection coefficient. Estimates of the selection coefficient for MO-10 varied from 0.19 to 0.32 ($s_x = 0.24$) indicating it was approximately 24% less fit than MS-1 under the selective conditions (Table 3). Variability between replications may have been due to undetected environmental differences between the growth chambers. Because of the large population size in each chamber (generally in excess of 10,000 colonies per generation), it is doubtful that random drift was a major problem in any of the mixtures.

Progeny were compared in three sets of mixtures, each replicated three times. Every set consisted of eight isolates, one each of the eight possible combinations of genes for virulence on wheat lines with *Pm*2, *Pm*3a, and *Pm*4. The progeny were selected at random with respect to virulence on CI 15889 and mating type because at the time of selection these characteristics were not known. Isolate frequency changes for a representative replication of one set of progeny is shown in Fig. 2. Estimates of isolate frequencies in the 8-way mixtures were imprecise due to the limited sample size (approximately 100 conidia per chamber per sampling time), which may account for some of the observed variability over time. The trends, however, were similar between replications. Average selection coefficients for the progeny are shown in Table 3.

The progeny selection coefficients were analyzed statistically (Table 4). No significant effects could be detected that were attributable to any of the identified virulence loci, either singly or in combination. The fitness of an isolate was also not correlated with the number of identified virulence genes it possessed ($r^2 = 0.001$). The average selection coefficients associated with alternate alleles

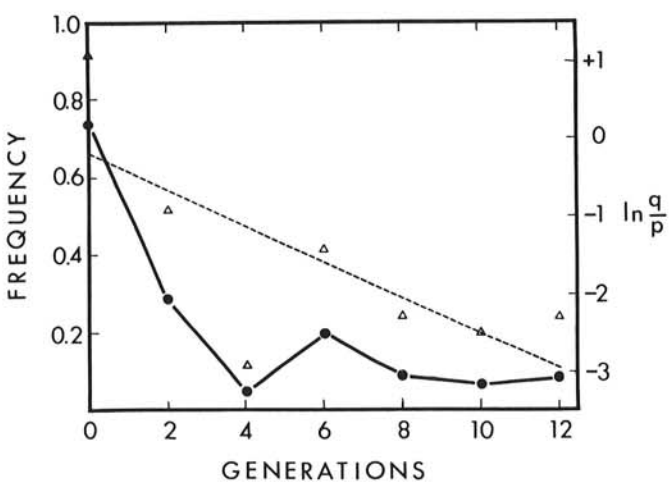

Fig. 1. Frequency of isolate MO-10 of *Erysiphe graminis* f. sp. *tritici* (closed circles) during twelve conidial generations in competition with MS-1 on the susceptible wheat line Chancellor. To estimate the selection coefficient, data were transformed to $\ln(q/p)$ (triangles) in which q is the frequency of MO-10 and p is the frequency of MS-1.

at the virulence loci are shown in Table 5. Isolates also varied in mating type; however, no difference in selection coefficients was associated with this locus.

DISCUSSION

In this study, segregational analysis was used to examine isolates of *E. g. tritici* for possible evidence of stabilizing selection. This required genetic analysis to determine the control of virulence. Isolates MS-1 and MO-10 were found to differ by at least four apparently unlinked genes for virulence, one controlling each difference in host range between the isolates. MS-1 was avirulent and MO-10 was virulent on wheat lines with the *Pm*2, *Pm*3a, and *Pm*4 resistance genes and on line CI 15889. These results are similar to those of Leijerstam (7), who reported single-gene control of virulence on lines with *Pm*2 and *Pm*4. For virulence on lines with *Pm*3a, he detected a single locus in two crosses and two loci in a third cross. One of these loci was apparently linked to the locus controlling virulence on *Pm*4.

MO-10 was clearly less fit than MS-1 under the environmental conditions used in this study. The average selection coefficient for MO-10 was 0.24. Since the four virulence genes in MO-10 were not required for it to infect the host line used in the fitness tests (cultivar Chancellor), these virulence genes were (by definition) unnecessary. Thus, the reduced fitness of MO-10 compared to MS-1 appeared to conform with Vanderplank's hypothesis that pathogens with unnecessary virulence genes are less fit than pathogens without such genes. However, when F_1 progeny of MS-1 and MO-10 were examined under the same environmental conditions, unnecessary virulence genes and reduced fitness were not associated. This indicated that segregation of genes controlling these traits had occurred and that most of the large difference in fitness between MS-1 and MO-10 was controlled by loci other than the unnecessary virulence genes.

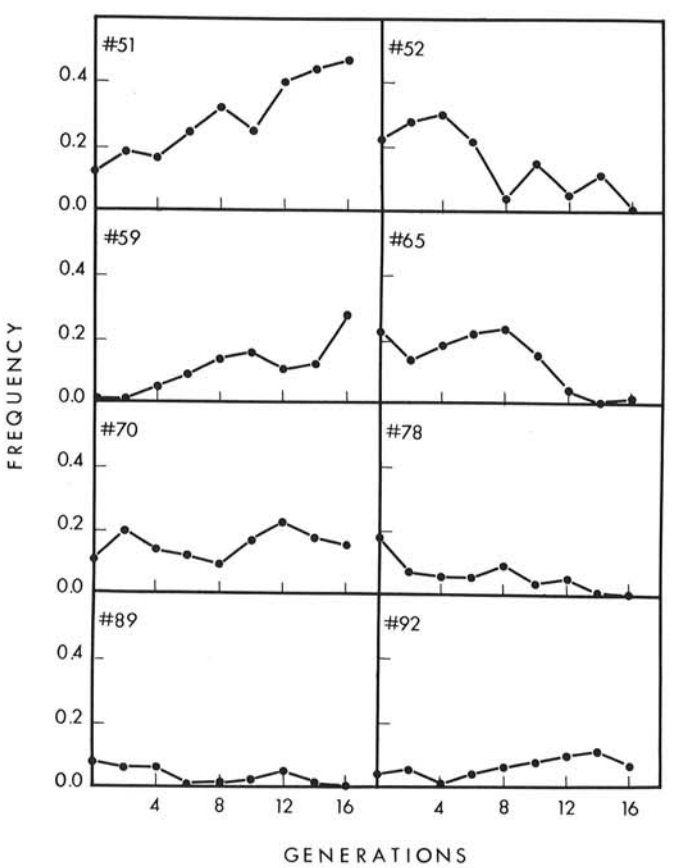

Fig. 2. Frequencies of eight isolates of *Erysiphe graminis* f. sp. *tritici* during sixteen conidial generations on the susceptible cultivar Chancellor (progeny set 1).

Although we found no evidence for stabilizing selection, Vanderplank's hypothesis was not disproven. It is possible that the fitness differences examined may have been controlled by undetected loci for virulence, although there is no evidence of this. It is also possible that the virulence genes studied have a small effect on fitness that could not be statistically detected in the presence of loci controlling large differences in fitness. In addition, according to stabilizing selection theory (20,21), not all unnecessary virulence genes must have a debilitating effect on the fitness of the pathogen. The hypothesized selective pressure against unnecessary virulence also need not exist in all environments or during all stages of pathogen's life cycle.

Segregational analysis is used to separate correlated phenomena, that is, to test whether certain differences in phenotype are attributable to specific genetic loci. The reasoning involved in the application of this technique to tests of the hypothesis of stabilizing selection is as follows: if reduced fitness and unnecessary virulence segregate from one another, reduced fitness and virulence can not be causally related. If segregation is not observed, fitness and virulence are either due to the same gene or closely linked genes. This approach to the study of stabilizing selection has certain advantages, but it also has definite limitations. An advantage is that by measuring the fitness of individual isolates, rather than the average fitness of isolates in an undefined mixture, even rare segregations can be detected. The method also requires only a single cross. However, it is reasonable to expect that there are many differences in fitness between pathogen isolates that are not controlled by virulence genes. Thus, it is likely that most segregation tests, as this one, will show that a large portion of the difference in fitness segregates from virulence. Segregational

TABLE 4. Analysis of variance for the average selection coefficients of the progeny of isolates MS-1 and MO-10 of *Erysiphe graminis* f. sp. *tritici*[a]

Source	Degrees of freedom	Mean square[b]
Progeny set	2	0.0040
*P*2	1	0.0104
*P*3a	1	0.0216
*P*4	1	0.0001
*P*2 × *P*3a	1	0.0006
*P*2 × *P*4	1	0.0193
*P*3a × *P*4	1	0.0008
*P*2 × *P*3a × *P*4	1	0.0000
Error	14	0.0111

[a]Progeny sets were treated as blocks and replicates as repeated determinations. Loci *P*15889 and *Mat* were not included in this analysis because they were detected after the experiment was designed. Student's *t*-tests, however, showed no difference in selection coefficients for alternate alleles at these loci.
[b]None of the values were significant.

TABLE 5. Average selection coefficients associated with alternate alleles at virulence loci in the progeny of isolates MS-1 and MO-10 of *Erysiphe graminis* f. sp. *tritici*[a]

Gene	Average selection coefficient[b]
*P*2	0.16
*p*2	0.11
*P*3a	0.10
*p*3a	0.16
*P*4	0.14
*p*4	0.13
*P*15889	0.15
*p*15889	0.12

[a]The average selection coefficients for *mat*+ and *mat*− were 0.13 and 0.14, respectively.
[b]None of the differences between alternate alleles were significant (see Table 4).

analysis as used here is inefficient at detecting small effects of virulence loci in such a situation. This is because of the limited number of progeny that can be reasonably tested. This inefficiency was clearly a problem in this study, as small differences in fitness (such as between *P*3a and *p*3a, Table 5), which may be very important in evolutionary terms, were judged not significant. However, if there is circumstantial evidence indicating that the difference in fitness between two isolates may be due to specific virulence loci, segregational analysis can provide a rapid test of the hypothesis.

LITERATURE CITED

1. Briggle, L. W. 1969. Near-isogenic lines of wheat with genes for resistance to *Erysiphe graminis* f. sp. *tritici*. Crop Sci. 9:70-72.
2. Bronson, C. R. 1981. The influence of unnecessary virulence genes on the reproductive fitness of *Erysiphe graminis* f. sp. *tritici*. Ph.D. thesis. Michigan State University, East Lansing. 86 pp.
3. Crill, P. 1977. An assessment of stabilizing selection in crop variety development. Annu. Rev. Phytopathol. 15:185-202.
4. Grant, M. W., and Archer, S. A. 1983. Calculation of selection coefficients against unnecessary genes for virulence from field data. Phytopathology 73:547-551.
5. Hiura, U. 1978. Genetic basis of formae speciales in *Erysiphe graminis* D.C. Pages 101-128 in: The Powdery Mildews. D. M. Spencer, ed. Academic Press, New York. 565 pp.
6. Kiyosawa, S. 1982. Genetics and epidemiological modeling of breakdown of plant disease resistance. Annu. Rev. Phytopathol. 20:93-117.
7. Leijerstam, B. 1972. Studies in powdery mildew on wheat in Sweden. III. Variability of virulence in *Erysiphe graminis* f. sp. *tritici* due to gene recombination and mutation. Statens Vaxtskyddsanst. Medd. 15:231-248.
8. Leonard, K. J. 1969. Selection in heterogeneous populations of *Puccinia graminis* f. sp. *avenae*. Phytopathology 59:1851-1857.
9. Leonard, K. J., and Czochor, R. J. 1980. Theory of genetic interactions among populations of plants and their pathogens. Annu. Rev. Phytopathol. 18:237-258.
10. Mac Key, J. 1973. Fitness of virulence genes in rusts and mildew in absence of host selection pressure. (Abstr.) Genetics 74 (June Suppl. No.2, Part 2):169.
11. Mac Key, J. 1981. Alternative strategies in fungal race-specific parasitism. Theor. Appl. Genet. 59:381-390.
12. Martin, T. J., and Ellingboe, A. H. 1976. Differences between compatible parasite/host genotypes involving the *Pm*4 locus of wheat and the corresponding genes in *Erysiphe graminis* f. sp. *tritici*. Phytopathology 66:1435-1438.
13. Masri, S. S., and Ellingboe, A. H. 1966. Germination of conidia and formation of appressoria and secondary hyphae in *Erysiphe graminis* f. sp. *tritici*. Phytopathology 56:304-308.
14. Mortensen, K. 1974. Konkurrenceforsog med isolater af meldugsvampen, *Erysiphe graminis*, med forskellige patogenitetsgener. Nord. Jordbrugsforsk. 56:387-388.
15. Moseman, J. G. 1959. Host-pathogen interaction of the genes for resistance in *Hordeum vulgare* and for pathogenicity in *Erysiphe graminis* f. sp. *hordei*. Phytopathology 49:469-472.
16. Moseman, J. G. 1973. Genetics of resistance to wheat powdery mildew. Pages 435-438 in: Proc. 4th Int. Wheat Genetics Symposium. E. R. Sears and L. M. S. Sears, eds., 6–11 August 1973. University of Missouri, Columbia.
17. Moseman, J. G., and Powers, H. R. Jr. 1957. Function and longevity of cleistothecia of *Erysiphe graminis* f. sp. *hordei*. Phytopathology 47:53-56.
18. Nass, H. A., Pedersen, W. L., MacKenzie, D. R., and Nelson, R. R. 1981. The residual effects of some "defeated" powdery mildew resistance genes in isolines of Chancellor winter wheat. Phytopathology 71:1315-1318.
19. Parlevliet, J. E. 1981. Stabilizing selection in crop pathosystems: An empty concept or reality? Euphytica 30:259-269.
20. Vanderplank, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York. 349 pp.
21. Vanderplank, J. E. 1968. Disease Resistance in Plants. Academic Press, New York. 206 pp.
22. Wolfe, M. S. 1973. Changes and diversity in populations of fungal pathogens. Ann. Appl. Biol. 75:132-136.
23. Wolfe, M. S., and Schwarzbach, E. 1978. Patterns of race changes in powdery mildews. Annu. Rev. Phytopathol. 16:159-180.